

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The specification is changed as follows:

Page 12, second paragraph, continuing to page 13, delete in its entirety and replace with the following new paragraph:

As stated above, the peptide may have the orientation of the formula given above or may have the retro orientation as a whole or in part. Any of the repetitive sequences can thus be in the retro orientation i.e. (A-C-B-A) or the orientation given in the formula, wherein the remaining groups are in the orientation as given in the formula. In a suitable embodiment as is apparent from the examples a peptide has the repetitive sequence (A-B-C-A) present in the retro orientation more often than in the orientation as presented in the formula, and/or at least one repetitive sequence has the orientation of the formula (ABCA) and at least one has the retro orientation (ACBA). It is to be noted that in repetitive sequences A, B and C may be different from each other, e.g. A₂ may [.]be Lys in the first sequence and Arg in the second, etc.

Examples of amphiphilic cationic peptides of the formula are:

- BP 1 Gly-Arg-Leu-Arg-Lys-Lys-Trp-Lys-Ala-Phe-Lys-Lys-Phe-Leu-Lys-Ile-Leu-Ala-Cys
[(sequence id. No. 1)] (SEQ ID NO. 1)
- BP2 Gly-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ala-Phe-Lys-Lys-Phe-Leu-Lys-Ile-Leu-Ala-Cys
[(sequence id. No. 2)] (SEQ ID NO. 2)
- BP2.3 Gly-Lys-Trp-Lys-Ala-Phe-Lys-Lys-Ala-Phe-Lys-Lys-Phe-Ala-Lys-Ile-Leu-Ala-Gly
[(sequence id. No. 3)] (SEQ ID NO. 3)
- BP2.4 Gly-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ala-Phe-Lys-Lys-Phe-Leu-Lys-Ile-Leu-Ala-Gly
[(sequence id. No. 4)] (SEQ ID NO. 4)
- BP2.5 Cys-(Gly)₉-Lys-Trp-Lys-Ala-Phe-Lys-Lys-Ala-Phe-Lys-Lys-Phe-Ala-Lys-Ile-Leu-
Ala-Cys-Gly [(sequence id. No. 5)] (SEQ ID NO. 5)

Examples of cationic peptides similar to those of the formula according to the invention are:

BP1.1 Gly-Lys-Leu-Lys-Lys-Lys-Trp-Lys-Ala-Ala-Lys-Lys-Phe-Leu-Lys-Lys-Cys-Ser
[(sequence id. No. 6)] (SEQ ID NO. 6)

BP2.1 Gly-Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ala-Ala-Lys-Lys-Phe-Leu-Lys-Lys-Cys-Ser
[(sequence id. No. 7)] (SEQ ID NO. 7)

BP2.2 Gly-Lys-Trp-Lys-Ala-Phe-Lys-Lys-Ala-Ala-Lys-Lys-Phe-Ala-Lys-Lys-Cys-Ser
[(sequence id. No. 8)] (SEQ ID NO. 8)

Page 14, first full paragraph on page 14, and continuing to page 15, delete in its entirety and insert the following new paragraph.

Bactericidal activity of the peptides of the invention was demonstrated by determining the effect on survival of representative Gram-negative (*Escherichia coli* serotype 0111:B4) and Gram-positive (*Staphylococcus aureus*) bacteria in liquid culture relative to a control without added peptide. A peptide encompassing a selected LPS-binding domain from a well characterized human antiendotoxin protein, known as bactericidal/permeability increasing protein (BPI), from amino acids 85 to 102, denoted as BPI₍₈₅₋₁₀₂₎, was included for a direct comparison of the efficacy of the endotoxin-binding domain of a naturally occurring protein and the synthetic peptides of the invention. The amino acid sequence of the BPI₍₈₅₋₁₀₂₎ peptide is:

Ile-Lys-Ile-Ser-Gly-Lys-Trp-Lys-Ala-Gln-Lys-Arg-Phe-Leu-Lys-Met-Ser-Gly-(Cys)

(SEQ ID NO. 9)

The BPI₍₈₅₋₁₀₂₎ reference peptide was synthesized as previously described for peptides of the invention and provided with a C-terminal Cys residue for the purpose of biotinylation as previously described. A dose-dependent reduction in the cell-density of both Gram-negative and

Gram-positive bacterial cultures was evident with BP1 and BP2, contrary to the BPI₍₈₅₋₁₀₂₎ peptide, which showed a slight effect on Gram-negative bacteria and no effect on Gram-positive bacteria (Table 1). Incubation of various concentrations of the synthetic peptides according to the invention for a limited time period on the survival of both types of bacteria again illustrated their potent anti-microbial activity as opposed to that of the BPI₍₈₅₋₁₀₂₎ peptide (Fig. 1) with the sequence corresponding to that of the potential LPS binding domain of the native BPI protein. This is indicative of a broad specificity of the synthetic peptides, in keeping with the general bactericidal properties of this class of linear amphipathic α -helical peptides. To demonstrate the influence of peptide sequence as defined by the formula of the invention on antibacterial properties, analogues were designed so as to contain subtle sequence differences compared with the representative BP1 and BP2 peptides of the invention. The absolute requirement for a tandem array of 2 or more of the repetitive sequence motifs $(A_2-B_2-C_1-A_3)_m$, flanked by a hydrophobic C-terminal domain $(C_2)_n$, was demonstrated with the use of the BP1.1, BP2.1 derivatives. The B₂ residue in the N-terminal repetitive sequence element in each instance, instead of an aromatic amino acid residue, as specified by the formula of the invention, was replaced with an Ala residue and the hydrophobic $(C_2)_n$ element made hydrophilic by substitution with Lys and Cys residues. Although the overall homology between these derivatives and that of the parent peptides according to the invention is high, no detectable anti-microbial activity of the derivatives was apparent even at the highest concentration tested (20 μ g/ml). The BP2.3, BP2.4 and BP2.5 peptides according to the invention displayed similar anti-microbial efficacy as the BP2 peptide under the same conditions (data not shown).

PRELIMINARY AMENDMENT
U.S. Appln. No. 09/493,211

IN THE CLAIMS:

The claims are amended as follows:

8. (Amended) The peptide BP 1, having [Sequence id. no.1] SEQ ID NO. 1.
9. (Amended) The peptide BP 2, having [Sequence id. no. 2] SEQ ID NO. 2.
10. (Amended) The peptide BP 2.3, having [Sequence id. no. 3] SEQ ID NO. 3.
11. (Amended) The peptide BP 2.4, having [Sequence id. no. 4] SEQ ID NO. 4.
12. (Amended) The peptide BP 2.5, having [Sequence id. no. 5] SEQ ID NO. 5.